REMARKS

1. Amendments to the claims

Claim 1 has been amended to specify that the claimed genetically modified plant is able to accumulate the heavy metals Zn, Co, Cd or Pb and translocate them to the shoots. Support for this amendment is found § [0027], [0028], [0049] and [0052] and in claim 11 of the present Application as published.

Claims 9 and 20 were discovered by Counsel to be improper multiple dependent claims, with 9 being multiply dependent on both 1 and 5, and 20 on 1, 5 and 6. Claims 9 and 20 have been amended to be singly dependent on 1, and to recite providing the vector. It is also specified that the cell or tissue is one the vector is capable of transforming.

New claims 35 and 36 have been added. Support for this amendment is found in Example 3, § [0154], of the present Application. While all the eukaryotic P_{1B}-type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass participate in the loading of heavy metal into the plant, it is preferable that, when overexpressed in a genetically modified plant as claimed, one of the P_{1B}-type ATPase enhances translocation of heavy metals from the roots to the upper parts of the plant and another P_{1B}-type ATPase favours vacuolar sequestration of said heavy metals.

New claim 37 has been added. Support for this amendment is found in Example 3, § [0154], of the present Application.

Claims 1, 2, 4, 7, 9, 10, 20, and 29-34 have been amended to correct grammatical errors and/or to comply with U.S. Patent practice.

2. Prior Art Issues

Claims 1, 2 and 4-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Borremans *et al.* (EP 1 136 558) in

view of Bernard *et al* (WO 2004/078905) and Chaney *et al.* (US 5,944,872).

The present invention as claimed relates to a genetically modified plant, comprising one or more than one copy of at least a two different sequences encoding two different **eukaryotic P_{1B}-type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass** and that it overexpresses said two different P_{1B}-type ATPase. This plant is able to accumulate the heavy metals Zn, Co, Cd or Pb and translocate them to the shoots.

Accordingly, the present invention as claimed is limited to:

- a **P_{1B}-type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass**, but not to any P-type ATPase and to any P_{1B}-type ATPase, such as a P_{1B}-type ATPase of the Cu^{2+} subclass, and
- an enzyme of **eukaryotic** origin, but not to an enzyme of prokaryotic origin for example.

The Examiner argues that Borremans is relied upon because it provides transformed plants expressing at least one heterologous P-type ATPase of eukaryotic origin. The Examiner also argues that Borremans provides the motivation to use P-type ATPase proteins encoding genes, including the *ZntA* gene to produce transgenic plants with heavy metal tolerance and accumulation activity (see the § between page 3 and 4 of the Office Action).

However, the ZntA P-type ATPase of *E. coli* is a prokaryotic P-type ATPase which pumps Zn^{2+}, Cd^{2+} and Pb^{2+} (see § [0021] of the present Application), but not an eukaryotic P_{1B}-type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass.

Moreover, Borremans was published in 2001 and does not disclose any experimental data showing that the expression of ZntA in a transgenic plant provides tolerance and accumulation of heavy metal and therefore showing that genetically modified plants expressing ZntA can be useful for phytoremediation of heavy metals from soil.

Enclosed PCT Application WO 02/081707 published in 2002 (see § [0021] of the present Application and the response to the 1st Office Action) teaches that genetically modified plants transformed with a recombinant vector comprising a sequence encoding a prokaryotic P-type ATPase, in particular *E. coli* ZntA protein exhibit an enhanced resistance to heavy metals (*i.e.*, lead and cadmium) but a **decreased uptake of heavy metals** compared to the wild-type plant (see page 4, line 1-6 and page 13, lines 17-24 of WO 02/081707). Therefore, said genetically modified plants overexpressing ZntA cannot be used for phyroremediation, since they do not accumulate heavy metals. As specified in the present Application (see § [0041]), *ZntA* (as well as homologous genes thereof), are not appropriate ATPases for use according to the invention.

In view of Borremans and WO 02/081707 and as stated by the Examiner, P-type ATPase genes provides tolerance only to heavy metals. However, in view of these documents, P-type ATPase genes do not provide heavy metal accumulation and translocation to the shoots.

The claimed genetically modified plants according to the present Application are able to accumulate the heavy metals Zn, Co, Cd or Pb and translocate them to the shoots (see § [0049] and [0052] and Example 3 of the present Application).

Hence, contrary to what is asserted by the Examiner (see page 4 of the Office Action), in view of the results described in the present Application, the Applicant provides unexpected results associated with the use of two different eukaryotic P_{1B}-type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass that could not have been obtained with the prokaryotic P-type ATPase genes as disclosed in Borremans and WO 02/081707, since the prokaryotic P-type ATPase genes do not provide heavy metal accumulation and translocation to the shoots.

In view of Borremans and WO 02/081707, the person skilled in the art would not have predicted the successful result of

overexpressing two different eukaryotic P_{1B}-type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass in a plant for phytoremediation, *i.e.*, a plant able to accumulate the heavy metals Zn, Co, Cd or Pb and translocate them to the shoots.

Bernard and Chaney do not suggest the specific combination of two different eukaryote P_{1B}-type ATPases of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass for producing a plant for phytoremediation. Further, Bernard does not provide any experimental results.

Therefore, none of the cited documents provides motivation for overexpressing two different eukaryote P_{1B}-type ATPases of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass in a genetically modified plant, for producing a plant able to accumulate the heavy metals Zn, Co, Cd or Pb and translocate them to the shoots.

In view of these cited documents, the person skilled in the art would have not be motivated to produce such a genetically modified plant.

Therefore the subject-matter of claims 1, 2 and 4-36 was not obvious over Borremans *et al.* in view of Bernard *et al* and Chaney *et al.*

Respectfully submitted,

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Enclosures
-WO 02/081707

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